

saturated with hydrogen bromide at room temperature was heated under reflux for 12 hours. The solution was extracted with 10% aqueous sodium carbonate, concentrated, and the residue was sublimed under reduced pressure. Recrystallization of the sublimate from 95% ethanol yielded 64.7 mg. (64.7%) of unchanged III, m.p. 105.5–106°. A solution of 0.10 g. of III and 0.1 g. of iodine in 50 ml. of benzene was heated under reflux for 10 hours, illuminated with a 275 watt General Electric sun lamp at a distance of 10 cm.

The iodine was removed by extraction with aqueous sodium bisulfite, and 78.2 mg. (78.2%) of III was recovered from the benzene solution by concentration and sublimation at 0.17 mm. with a heating block temperature of 120°. Similar results were obtained by heating 0.20 g. of III and a solution of 1.0 g. of hydrogen bromide in 50 ml. of  $\alpha$ -picoline under reflux for 144 hours. The recovery of unchanged III was 169 mg. (84.5%).

CAMBRIDGE, MASSACHUSETTS RECEIVED AUGUST 31, 1950

[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY,<sup>2</sup> FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

## Structural Aspects of the Color Reaction of Lignin with Phenols<sup>1</sup>

BY JOHN C. PEW

The chemistry of the long-known color reaction of lignin with phenols has been investigated. The absorption spectra of the color resulting from the action of resorcinol on spruce "native" lignin in acid solution was found to have a maximum at identical wave length to that of the color resulting from the acidification of catalytically reduced 4',7-dihydroxy-3'-methoxyflavanone. This is also identical to the maxima obtained by reduction of 2',4,4'-trihydroxy-3-methoxychalcone with lithium aluminum hydride or by the condensation of coniferylaldehyde with resorcinol in acid solution. With aspen "native" lignin the maximum absorption is between that of reduced 4',7-dihydroxy-3'-methoxyflavanone and reduced 4',7-dihydroxy-3',5'-dimethoxyflavanone. With spruce and aspen wood the resorcinol color corresponds to the condensation product of resorcinol with coniferylaldehyde substituted in the 5 position with a methoxyl or propenyl group.

Although the color reactions of lignin with phenols have been known a long time<sup>3,4</sup> and the literature on the subject is rather extensive, the chemistry involved has not been clearly established. The phenol reagent most used as a color test for lignin has been phloroglucinol and hydrochloric acid (Wiesner reagent). Much confusion has resulted from claims that numerous substances (coniferin, vanillin, ferulic acid, aliphatic aldehydes, clove oil, sassafras oil, cinnamaldehyde, piperonal, salicylaldehyde, hydroxyfurfural, eugenol, glucoseen, etc.) give a "lignin reaction" with this solution. Actually, in many cases the colors produced are not even visually similar to that obtained with lignin; in other instances, impurities in the compounds are responsible. Thus it is easily demonstrated that vanillin gives an orange color with the phloroglucinol reagent rather than the purple-red color produced by wood. Klason<sup>5</sup> has shown that coniferin and coniferyl alcohol, when purified, no longer give the color and Herzog and Hillmer<sup>6</sup> indicated that isoeugenol gives a positive reaction only after auto-oxidation. Recently Adler, Björkqvist and Häggroth<sup>7</sup> showed that eugenol, which gives color reactions with phenols that are very similar to those obtained with lignin, contains coniferyl aldehyde and its methyl ether, which are responsible for the reaction, and also confirmed the fact that pure coniferyl alcohol is without action.

A further complication arises in the claim<sup>8,9</sup> that the color reactions are not due to the lignin, but rather to "traces of materials of aldehydic

nature which always accompany lignin." Brauns,<sup>10</sup> however, concluded that the phloroglucinol color reaction is a fundamental property of spruce wood and this view appears to be currently accepted.

In spite of the rather extensive work on the subject, few investigators have even hazarded a guess as to the chemical structure of the chromophoric group developed. Hagglund and Johnson<sup>11</sup> claimed that a free hydroxyl group must be present in the phenol used and that a semi-acetal is produced. References to aldehydic groups and coniferylaldehyde as the causative agents are, however, fairly numerous.

Although the groups in lignin responsible for this color reaction probably form only a small part of the molecule (Adler and Ellmer<sup>12</sup> estimate one group to between 40 and 60 phenylpropane units), it was hoped that the placing of the reaction on a structural basis might give information on linkages within the main portion of the compound. A means of attacking the problem of structure was found some years ago in the observation that the reduction of properly substituted flavanones gave colors strikingly similar to those produced by wood with the appropriate phenol. Investigation of the literature at that time revealed that, although the flavanones were well established in structure, the chemistry of their reduction products was in an unsatisfactory state. The work of Geissman and Clinton<sup>13</sup> clarified this matter.

### Results

Brauns' spruce "native" lignin was chosen as the first substance to investigate, since it is soluble in alcohol, gives the color reaction readily, and, if not identical with the bulk of the lignin in wood, probably is very closely allied to it. Figure 1 gives the absorption spectra produced by the re-

(1) Presented before the American Chemical Society, Chicago, Ill., September 3-8, 1950. Article not copyrighted.

(2) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

(3) F. F. Runge, *J. prakt. Chem.*, **1**, 24 (1834).

(4) J. Wiesner, *Akad. Wiss. Wien*, **77**, 60 (1878).

(5) P. Klason, *Ber.*, **62**, 635 (1929).

(6) R. O. Herzog and A. Hillmer, *ibid.*, **62**, 1600 (1929).

(7) E. Adler, K. J. Björkqvist and S. Häggroth, *Acta Chem. Scand.*, **2**, 93 (1948).

(8) E. C. Crocker, *Ind. Eng. Chem.*, **13**, 625 (1921).

(9) K. Wiechert, *Papier Fabr.*, **37**, 17, 30 (1939).

(10) F. E. Brauns, *THIS JOURNAL*, **61**, 2121 (1939).

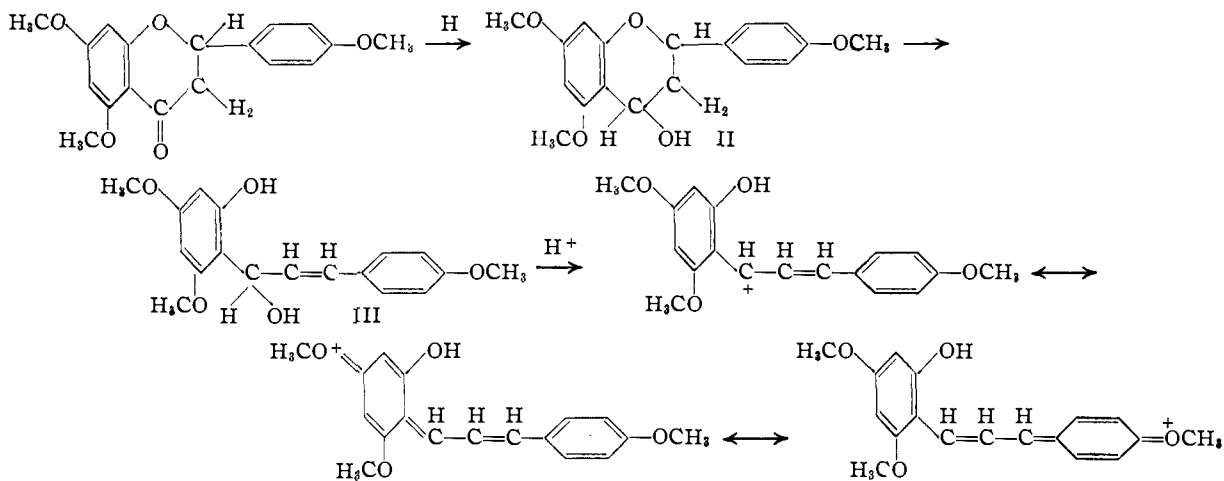
(11) E. Hagglund and T. Johnson, *Biochem. Z.*, **187**, 98 (1927).

(12) K. Adler and L. Ellmer, *Acta Chem. Scand.*, **2**, 839 (1948).

(13) T. A. Geissman and R. O. Clinton, *THIS JOURNAL*, **68**, 700 (1946).

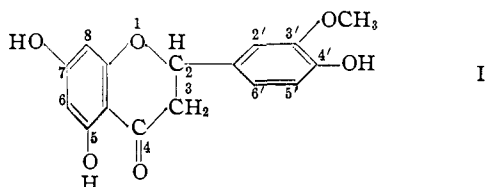
action of spruce "native" lignin with phenols in acid solution, and of certain catalytically reduced

amalgam<sup>14</sup> reduction, followed by acidification with a strong acid, takes the course<sup>13,14</sup>



flavanones. The "native" lignin-phloroglucinol curve A has a maximum at slightly lower wave length than that of curve B, resulting from the acidification of reduced homoeriodictyol (4',5,7-trihydroxy-3'-methoxyflavanone), I.

With this flavanone the corresponding 4-hydroxyflavane II was isolated, although with other substituted flavanones isolation was unsuccessful.



With the 4'-methyl ether of the latter compound the maximum is still further shifted toward the region of lower wave length as shown in curve C. The native "lignin" also reacted with trimethoxyphloroglucinol to give a curve (not plotted) with a maximum at 568 m $\mu$ . This demonstrates that, contrary to the claim of Hagglund and Johnson,<sup>11</sup> the presence of a free hydroxyl group in the phenol is not necessary to develop color.

To establish further the similarity of the lignin-phenol colors to those of reduced flavanones, since the curves are quite regular and without points of inflection, resorcinol was used as the phenol with "native" lignin to produce curve D, possessing a maximum at identical wave length to that of 4',7-dihydroxy-3'-methoxyflavanone, curve E. Since the use of resorcinol as contrasted to phloroglucinol appears to give curves with sharper maxima and shifts the maxima toward the longer wave lengths, thus placing them further away from the region where lignin itself absorbs, this phenol was used in most of the succeeding work. It should be noted that although the maximum obtained with the lignin-resorcinol color is at the same wave length as that of the corresponding reduced flavanone, the former gives relatively higher values on either side of the maximum, especially in the low wave-length field.

The question immediately arises as to the structure of the reduced flavanones. Geissman and Clinton's work indicated that catalytic or sodium

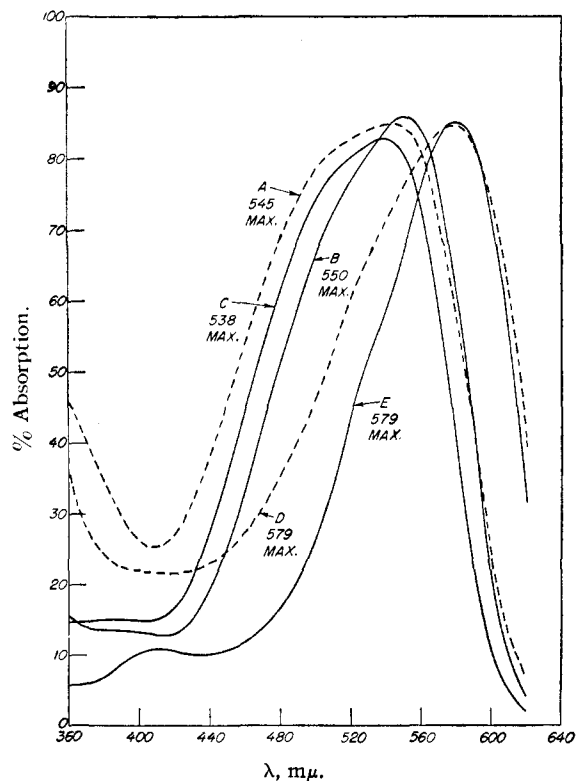


Fig. 1.—Absorption spectra of the reaction products of spruce native "lignin" with phloroglucinol and resorcinol and of certain catalytically reduced flavanones; solvent, 1 volume concentrated hydrochloric acid and 2 volumes 95% ethanol: A, spruce native lignin-phloroglucinol; B, reduced homoeriodictyol; C, reduced homoeriodictyol-4'-methylether; D, spruce "native" lignin-resorcinol; E, reduced 4',7-dihydroxy-3'-methoxyflavanone.

(14) Geissman and Clinton also postulated colored ions without ring opening. We believe that visible color is due essentially to the open formula.

The next question to be answered is the nature of the group in lignin which might give a structure similar to III. It is obvious such would result from a simple phenol-aldehyde condensation, as indicated in the reaction between resorcinol and coniferylaldehyde

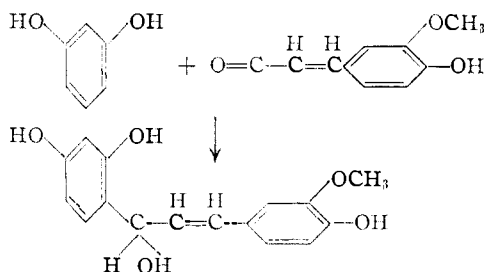


Figure 2, curve F, shows the color produced in this way in comparison with the corresponding reduced flavanone, curve E. It has also been found that

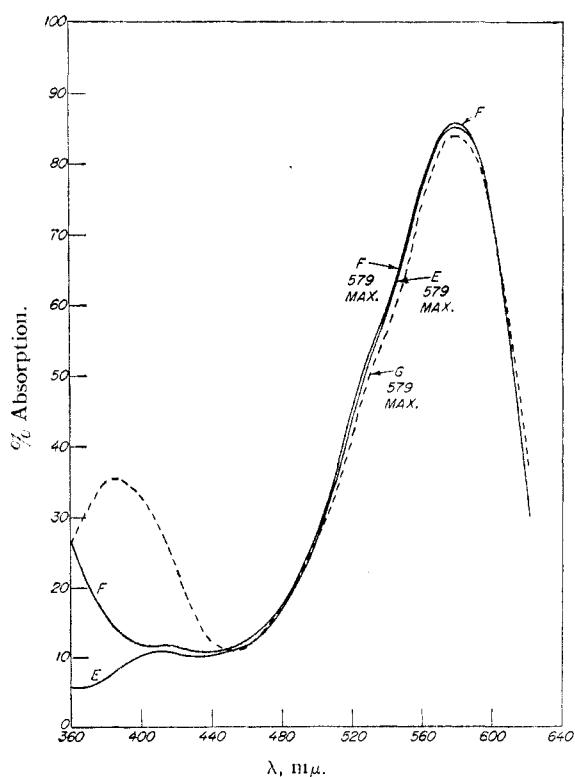
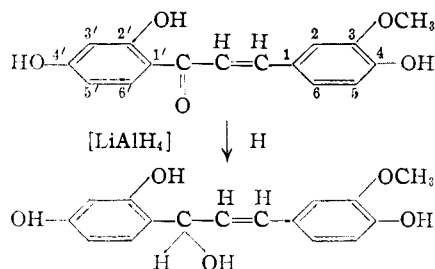


Fig. 2.—Absorption spectra of the coniferylaldehyde-resorcinol condensation product (F) compared with reduced 4',7-dihydroxy-3'-methoxyflavanone (E) and reduced 2',4,4'-trihydroxy-3-methoxychalcone (G): solvent, 1 volume concentrated hydrochloric acid, 2 volumes 95% ethanol in F and E, and 1 volume of a solution containing 38 parts hydrogen chloride in 62 parts 95% ethanol, 2 volumes 95% ethanol in case of G.

chalcones can be reduced with lithium aluminum hydride and thus three methods are available for producing colored ions for comparison with the lignin-phenol colors. Curve G shows the color produced by acidification of the product from the above reaction. The parallelism between the reduced flavanone colors and the phenol-aldehyde



condensation was proven in other instances as shown in Table I.

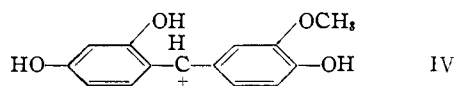
TABLE I

COMPARISON OF SPECTRA OF REDUCED FLAVANONES AND CORRESPONDINGLY SUBSTITUTED PHENOL-CINNAMALDEHYDE CONDENSATION PRODUCTS

Flavanone substituents						Phenol-cinnamaldehyde <sup>a</sup>	Flavanone <sup>a</sup>	Flavanone <sup>a</sup>
5	7	2'	3'	4'	5'	Max. mμ	Max. mμ	Max. mμ
		OH				480	480	
		OH		OCH <sub>3</sub>		562 <sup>b</sup>	557	
		OH		OCH <sub>3</sub>		531 <sup>b</sup>	528	
		OH	OH	OCH <sub>3</sub>	OH	550	550	
		OH	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	539	539	
		OH		OCH <sub>3</sub>	OCH <sub>2</sub> CH <sub>3</sub>	578	578	
		OH	OH	OCH <sub>3</sub>	OCH <sub>2</sub> CH <sub>3</sub>	545 <sup>c</sup>	545	
		OH		OCH <sub>3</sub>	OH	593	592	

<sup>a</sup> Solvent, except where indicated, 1 volume concentrated hydrochloric acid, 2 volumes 95% ethanol. <sup>b</sup> Data of Geissman in dioxane solvent, footnote (13). <sup>c</sup> Reduced chalcone.

It was further found that substituted benzaldehydes condensed with phenols to give an analogous series of colored compounds. Thus vanillin and resorcinol give the resonating ion IV containing



one pair of carbon atoms less than the corresponding cinnamaldehyde series. The maximum produced is at the same wave length as that obtained by catalytic reduction of the appropriate benzophenone. Results with vanillin and other substituted benzaldehydes are included in Table II. This series was useful in predicting the effects of substitution in the less available cinnamaldehyde series.

Since the guaiacyl nucleus is known to occur in spruce wood, coniferylaldehyde and the 4'-hydroxy-3'-methoxyflavanones were first chosen for color comparison purposes. It does not necessarily follow that the color-forming groups in spruce lignin are correspondingly substituted and, therefore, a study was made of the effect varying the substituent groups in the colored substances. The results are shown in Table II.

It should be noted that the 4-hydroxy-3-methoxy product has a maximum at practically the same wave length as the methoxyl-free compound and that a similar situation exists with the 4-hydroxy-3,5-dimethoxy-material. Also conversion of the 4-hydroxy-3-methoxy-compound to the 3,4-dimethoxy compound causes a considerable shift in maximum toward the short wave-length region,

TABLE II  
EFFECT OF SUBSTITUENT GROUPS ON THE ABSORPTION SPECTRA OF CERTAIN COLORED IONS

Substituents			Max. m $\mu$	Max. m $\mu$
2	3	4		
		OH	480 <sup>a,b</sup>	402 <sup>d</sup>
		OCH <sub>3</sub>	562 <sup>a</sup>	492
OH			557 <sup>b</sup>	485
	OH	OH	490	525
	OCH <sub>3</sub>	OH	579 <sup>a</sup>	523 <sup>h</sup>
	OCH <sub>3</sub>	OCH <sub>3</sub>	578 <sup>a,b,c</sup>	517
	OCH <sub>3</sub>	OC <sub>2</sub> H <sub>5</sub>	568 <sup>b</sup>	520
	OCH <sub>3</sub>	OC <sub>2</sub> H <sub>5</sub>	573 <sup>a</sup>	
	OCH <sub>3</sub>	OCH(CH <sub>3</sub> ) <sub>2</sub>	578 <sup>b,e</sup>	523
	OCH <sub>3</sub>	OC <sub>6</sub> H <sub>5</sub>		522
		O-CH <sub>2</sub> -O		515
OH	OCH <sub>3</sub>		545 <sup>b,e</sup>	415
OCH <sub>3</sub>	OCH <sub>3</sub>			408
OCH(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>		498 <sup>b</sup>	410
OH		OH		518
	OH	OH	594 <sup>a</sup>	
	OCH <sub>3</sub>	OH	593 <sup>a,b</sup>	543
	OCH <sub>3</sub>	OH	588 <sup>a</sup>	535
	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	<sup>d</sup>	533
	OCH <sub>3</sub>	CH=CH=CH <sub>2</sub>	593 <sup>e,f</sup>	543
	OCH <sub>3</sub>	CH=CH-CH <sub>3</sub>		517 <sup>g</sup>
	OCH <sub>3</sub>	C=O(CH <sub>3</sub> )		

<sup>a</sup> Ion from catalytically reduced flavanone. <sup>b</sup> Ion from resorcinol-substituted cinnamaldehyde condensation product. <sup>c</sup> Substitution of phloroglucinol for resorcinol gave 550 m $\mu$ . <sup>d</sup> Ion from hydrogenation (2 mol.) of respective propenyl flavanone. <sup>e</sup> Ion from chalcone reduced with lithium aluminum hydride. <sup>f</sup> Ion produced by reduction of the flavanone with sodium-amalgam. <sup>g</sup> Solvent, 1 volume of a solution containing 38 parts hydrogen chloride in 62 parts ethanol, 2 volumes ethanol; in all other instances solvent was 1 volume concentrated hydrochloric acid, 2 volumes 95% ethanol. <sup>h</sup> Substitution of phloroglucinol for resorcinol gave 490 m $\mu$  in solvent described first in note (g).

while an ethoxy group in the 4-position has less effect and the isopropoxy group causes no shift.

The next material investigated was aspen "native" lignin. The absorption maximum obtained with resorcinol was at a longer wave length than that of coniferylaldehyde with this phenol but shorter than that of sinapaldehyde (4-hydroxy-3,5-dimethoxycinnamaldehyde), the presence of which grouping might be suspected from the known chemistry of hardwoods. A mixture of the two colors (as represented by their reduced flavanone equivalents) gave a curve very similar and with the maximum at nearly identical wave length to the lignin-resorcinol product as shown in Fig. 3.

In Fig. 4 the spectra of the colors developed by resorcinol and acid in wood sections (using unstained sections as blanks) are shown, curve K being spruce and curve L aspen. The results are surprising in that the maximum is at identical wave length in the two species and coincides with that of the sinapaldehyde-resorcinol color (and the correspondingly substituted reduced flavanone). Since no syringyl groups were found in spruce by Creighton and Hibbert<sup>15</sup> and syringaldehyde has never been detected in the commercial production of vanillin from sulfite waste liquors (resulting from the pulping of conifers only),<sup>16</sup> another explanation

(15) R. H. J. Creighton and H. Hibbert, *THIS JOURNAL*, **66**, 37 (1944).

(16) J. L. Salvesen, private communication.

was sought. As indicated in Table II, the substitution of the propenyl group for one of the methoxyl groups in the syringyl nucleus does not shift the position of the absorption maximum in the colored ions described. Curve I is the catalytically reduced 4',7-dihydroxy-3,5-dimethoxyflavanone and curve M 2',4,4'-trihydroxy-3-methoxy-5-propenylchalcone reduced with lithium aluminum hydride.

As with "native" lignin, the curves obtained from wood matched well the position of the maximum of the appropriate known compound but had higher relative absorption in the field of either side. Considerable effort was made to find the cause of this difference, particularly with the spruce native lignin. At first it was believed that a portion of the color-forming groups might be substituted benzaldehydes, rather than cinnamaldehydes. The color, using a mixture of coniferylaldehyde and vanillin with resorcinol, better approximated the spruce "native" lignin-resorcinol curve, without displacing the maximum. When the experiment was repeated with phloroglucinol as the phenol, however, the vanillin contribution faded out when the alcoholic solution was diluted with water according to the established procedure. Various mixtures of substituted cinnamaldehydes were next employed and a combination did, indeed, closely match the "native" lignin curve but for several rea-

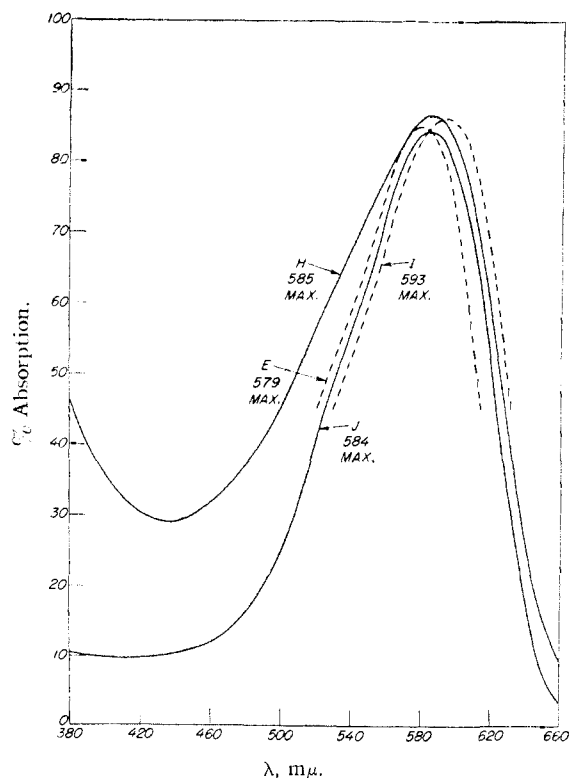


Fig. 3.—Absorption spectra of the reaction product of aspen "native" lignin with resorcinol (H) compared with reduced 4,7-dihydroxy-3'-methoxyflavanone (E), reduced 4,7-dihydroxy-3',5'-dimethoxyflavanone (I), and a mixture of the two reduced flavanones (J): solvent, 1 volume concentrated hydrochloric acid, 2 volumes 95% ethanol.

sons it seemed dubious that this particular mixture was actually involved in the case of the "native" lignin.

Several other lignin and lignin-like materials were examined for their color reactions with resorcinol. "Native" lignin from Scotch pine<sup>17</sup> gave a curve practically identical with that of spruce "native" lignin over the entire curve but had a maximum at a trifle shorter wave length (576 mμ). The "native" lignin from the same species after decay with *Lentinus lepidus* (which resulted in an approximately twofold increase in yield) gave a maximum at 582 mμ, that is, in the direction of wood itself. A sample of lignin obtained in 1.7% yield by the pressure methanol extraction<sup>18</sup> of insignis pine, previously extracted with ethanol-benzene, resulted in the same value. The results with a sample of the polymer resulting from the Fries reaction on vanillin acetate<sup>19</sup> are shown in Fig. 5. It is evident that the color-producing groups are C<sub>6</sub>-C in character rather than C<sub>6</sub>-C<sub>3</sub>. It also appears that the phenone group in the proposed monomer has been altered and, from the intensity of the color, aldehyde groups would seem quite frequent. These indications are not in accord with the formulation which has been proposed for this polymer.

(17) W. J. Schubert and F. F. Nord, *THIS JOURNAL*, **72**, 977 (1950).

(18) D. E. Bland, E. A. Hanson, C. M. Stewart and A. J. Watson, *J. Council Sci. Ind. Research*, **20**, 553 (1947).

(19) A. Russell, *THIS JOURNAL*, **70**, 1060 (1948).

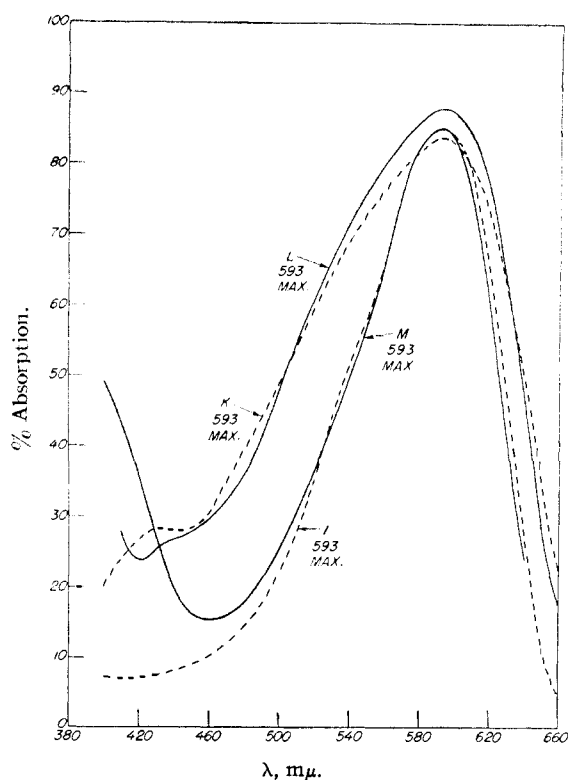
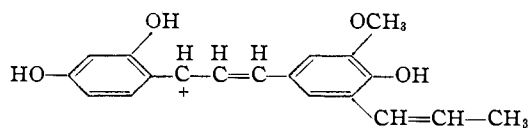
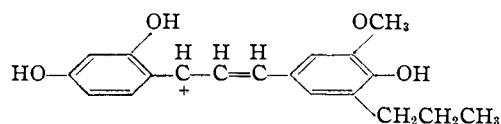


Fig. 4.—Absorption spectra of reaction product of spruce wood sections (K) and aspen wood sections (L) with resorcinol compared with reduced 4',7-dihydroxy-3',5'-dimethoxyflavanone (I), and reduced 2',4,4'-trihydroxy-3-methoxy-5-propenylchalcone (M): solvent, 1 volume concentrated hydrochloric acid, 2 volumes 95% ethanol.

On the other hand, the polymer obtained by Freudenberg<sup>20</sup> by the action of mushroom enzyme on coniferyl alcohol, which polymer is said closely to resemble natural lignin in all respects except for its solubility in organic solvents, approaches that of wood in the resorcinol color reaction. In Fig. 6, curve S of the polymer is compared to curve U, the ion



and to curve T, the ion



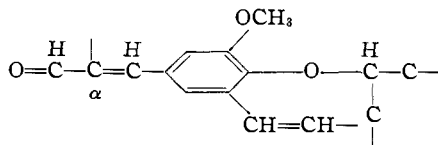
The polymer has the maximum at the same wave length as the latter curve and, of particular interest, diverges from the curve of the comparison substance in a manner similar to natural lignins.

Finally, purified spruce thioglycolic acid lignin prepared according to the modification of Brauns<sup>21</sup> also gives a curve (V, Fig. 6) similar to that of wood, but with a maximum at 589 mμ rather than 593 mμ.

(20) K. Freudenberg, *Proc. Heidelberg Acad. Science*, (1949), fifth paper.

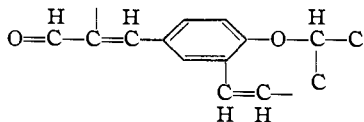
(21) F. Brauns, *THIS JOURNAL*, **61**, 277 (1939).

From the knowledge so far gained, it might be postulated that the structure of the group in spruce wood giving color with phenols (when in the presence of hydrochloric acid) is

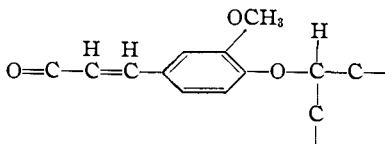


That the 4-hydroxyl may not be free is supported by the absence of a shift to a shorter wave length in the absorption maximum of the resorcinol developed color by prior treatment of the wood section with diazomethane in ether solution. It cannot be concluded that the  $\alpha$  carbon is unsubstituted, since catalytically reduced homoeriodictyol (max. 550  $m\mu$ ) and the corresponding 3-hydroxyflavanone reduced with granulated zinc and hydrochloric acid (max. 550  $m\mu$ ) give essentially identical curves.

The above assumption makes the formulation of color-producing groups in spruce "native" lignin somewhat difficult. They might be assumed thus



Such groups might be expected to give *p*-hydroxybenzaldehyde on nitrobenzene oxidation, which compound has not been reported with spruce. However, Bland and Cohen<sup>22</sup> in recent work indicate this aldehyde to be obtained in small amount by nitrobenzene oxidation of 7 out of 8 Australian softwoods tested. On the other hand, Adler and Ellmer<sup>13</sup> obtained coniferylaldehyde from wood (in extremely small yield) by extraction of the wood with tin chloride, which would suggest the unit to be



A third possibility is that of coniferylaldehyde attached at the 5-position by a group which exerts negligible effect on the location of the maximum (in respect to wave length) of the color produced by resorcinol. As indicated in Fig. 5, introduction of an acetyl group in the 5-position in vanillin causes a shift in the maximum of the resorcinol color in the direction of lower rather than higher wave length.

A fourth possibility is that of a coniferylaldehyde group attached at the 6-position.

### Experimental

**Synthesis of Chalcones and Flavanones.**—Chalcones were prepared by the cold or hot condensation methods outlined by Geissman and Clinton.<sup>23</sup> Frequently, it was necessary to alter the concentration of the alkali, the proportions of alkali and ethanol, or the temperature in order to obtain satisfactory results. Yields were often low. In obstinate

(22) D. E. Bland and W. E. Cohen, unpublished work.

(23) T. A. Geissman and R. O. Clinton, *THIS JOURNAL*, **68**, 697 (1946).

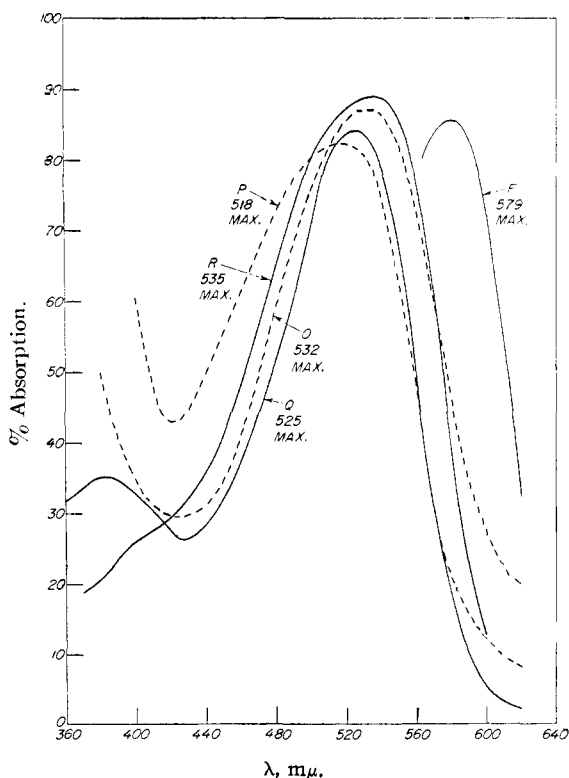


Fig. 5.—Absorption spectra of the color produced by the reaction of resorcinol with the polymer resulting from the Fries reaction on vanillin acetate compared to those of resorcinol with several possibly related substances: O, polymer; P, 2-hydroxy-3-methoxy-5-formylacetophenone; Q, vanillin; R, 5-propylvanillin; F, coniferylaldehyde; solvent, O, P, Q, 1 volume concentrated hydrochloric acid, 2 volumes 95% ethanol, R, F, 1 volume of a solution containing 38 parts hydrogen chloride in 62 parts 95% ethanol, 2 volumes 95% ethanol.

cases Russell's method<sup>24</sup> was used. Chalcones were generally isomerized to the flavanones by the acid method,<sup>23</sup> the alkaline method<sup>23</sup> being used in one or two instances.

**Reduction of Flavanones.**—These were catalytically reduced by a modification of the method of Geissman and Clinton.<sup>13</sup> One hundred mg. of the flavanone was dissolved in 25 cc. of ethanol in a 50-cc. volumetric flask, 100 mg. of Adams platinum oxide added, and the mixture hydrogenated at room temperature and 1 atm. pressure until 1.05 to 1.10 moles of hydrogen were absorbed. The solution was made up to volume with ethanol and filtered. Portions of this stock solution were further diluted to supply material for development of color. The 2,4,4'-trihydroxy-3'-methoxybenzophenone was similarly treated.

Although the ethanol solutions of the reduced flavanones appeared stable, they were stored in a refrigerator. One of the solutions was observed to give color in undiminished intensity after being stored in this manner for more than a year.

In the instance where the flavanone was reduced by sodium-amalgam reduction, the method described in the above reference was employed.

**Reduction of Chalcones with Lithium Aluminum Hydride.**—To a solution of 0.02 millimole of the chalcone in 10 cc. of absolute ether contained in a 25-cc. erlenmeyer flask, a piece of lithium aluminum hydride amounting to about 0.05 millimole was added. The flask was stoppered and shaken for 15 minutes. If the hydride had not all dissolved, it was crushed and shaking was continued until solution was complete. The flask was then allowed to stand for one hour, water added, followed by a drop of concentrated hydro-

(24) A. Russell, *J. Chem. Soc.*, 218 (1934); A. Russell and J. Todd, *ibid.*, 1066 (1934); 421 (1937).

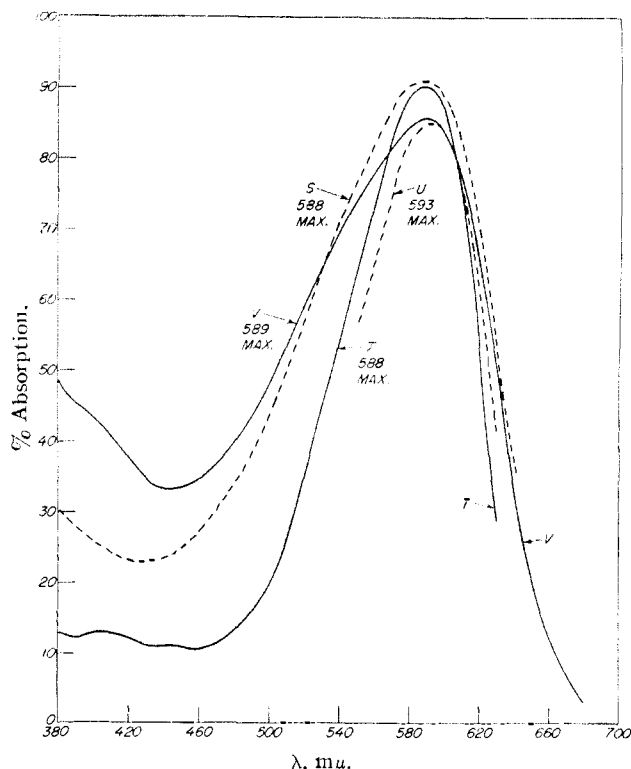


Fig. 6.—Absorption spectra of the reaction product of resorcinol with the polymer obtained by Freudenberg by polymerizing coniferyl alcohol with mushroom enzyme (S), and of resorcinol with spruce thioglycolic acid lignin (V) compared with reduced 4',7-dihydroxy-3'-methoxy-5'-propenylflavanone (U) and reduced 4',7-dihydroxy-3'-methoxy-5'-propylflavanone (T).

chloric acid. The ether layer was separated, the aqueous layer extracted with additional ether, and the combined ether extracts washed with 2 cc. of 2% aqueous sodium bicarbonate solution. The ether layer was superficially dried with anhydrous sodium sulfate and the ether removed under diminished pressure. The residue was dissolved in 20 cc. of 95% ethanol.

**Synthesis of Substituted Cinnamaldehydes.**—The *p*-methoxycinnamaldehyde was synthesized as described by Scholtz and Wiedemann,<sup>25</sup> coniferylaldehyde by the method of Pauley and Feuerstein,<sup>26</sup> and the 3,4-dimethoxy compound according to Feuerstein.<sup>27</sup> With the *o*-coniferylaldehyde, modifications of the procedure described by Pauly and Wascher<sup>28</sup> were used and with sinapaldehyde, modifications of Pauly and Strassberger's<sup>29</sup> synthesis. In these instances the methoxymethyl ether of the respective substituted benzaldehydes was prepared by refluxing the potassium salt with chloromethyl ether in anhydrous ether solution overnight. After adding water, removing the ether layer, washing the ether layer thoroughly with aqueous sodium hydroxide and drying, the methoxymethyl compound was crystallized from the ether at  $-30^{\circ}$ . The methoxymethyl cinnamaldehydes were also separated from the condensation mixtures by crystallization from cold ether rather than by vacuum distillation. The new cinnamaldehydes (3-methoxy-4-isopropoxy and 2-isopropoxy-3-methoxy) were prepared substantially according to the method for the 3,4-dimethoxy compound.<sup>27</sup>

**Synthesis of Substituted Benzaldehydes.**—The substituted benzaldehydes used for chalcone synthesis or for producing phenol-aldehyde colors were mostly available. The 3,4,5-trihydroxy compound was prepared according to Rosenmund<sup>30</sup> and the syringaldehyde as described by

Pearl.<sup>31</sup> Vanillin ethyl ether, isopropyl ether, and *o*-vanillin isopropyl ether were prepared in the usual manner by refluxing the potassium salt of the aldehyde with the respective alkyl iodide in anhydrous ethanol solution. The 5-allylvanillin was prepared according to Claisen and Eisleb<sup>32</sup> and 5-propylvanillin as described by Freudenberg and Richtzenhain.<sup>33</sup> The 5-propenylvanillin was prepared as follows:

To 50 g. of 5-allylvanillin were added 120 g. of potassium hydroxide dissolved in 150 cc. of methanol. The mixture was refluxed 6 hours with occasional thorough mixing, diluted with 500 cc. of water, and the solution poured into a solution of 300 cc. of acetic acid in 700 cc. of water. After standing overnight the crystals were filtered off, dried, and recrystallized from benzene, yielding 31 g. of the propenyl compound. Recrystallization from ethanol gave colorless needles, m.p. 98–99°.

**Development of Color with the Flavanones.**—From 0.5 to 2.0 cc. of the ethanol solution of the reduced flavanone containing sufficient material to give a maximum absorption of 80 to 90% (equivalent to 56 micrograms of the unreduced substance in the case of the reduced 4,7-dihydroxy-3'-methoxyflavanone, for example) was added to 5 cc. of concentrated hydrochloric acid and allowed to stand at room temperature 5 to 10 minutes to develop the color. The solution was then diluted to 15 cc. with ethanol, cooling under the tap.

**Development of Color with Phloroglucinol or Resorcinol and Substituted Cinnamaldehydes.**—These colors were produced by adding hydrochloric acid to equimolar quantities of the phenol and aldehyde in ethanol solution. Since the results were sometimes poor by using the concentrated acid, a solution of hydrogen chloride in 95% ethanol equivalent in strength to the concentrated acid was substituted. After development of the color, water was added to give a mixture equivalent to that which would have been obtained by the use of concentrated acid. As a typical example, 5 cc. of a 38% solution of hydrogen chloride in ethanol was added to a mixture of 0.85 cc. of 0.0005 molar ethanol solution of resorcinol and 0.85 cc. of 0.0005 molar ethanol solution of coniferylaldehyde. After standing at room temperature for ten minutes the mixture was diluted to 15 cc. with 3.8 cc. of water and the balance ethanol, undue temperature rise being prevented by cooling under the tap.

**Development of Color with Phloroglucinol or Resorcinol and Substituted Benzaldehydes.**—These were treated, in general, as with the cinnamaldehydes. The colors tended to fade rather badly, making accurate readings difficult. However, it was found that an excess of the phenol materially stabilized the colors, so that 2 moles of the phenol were used to 1 mole of the aldehyde. In some instances the color disappeared entirely or was greatly diminished when water was added to the reaction mixture according to the method used with the substituted cinnamaldehydes. In these cases dilution was made with 95% ethanol only. It was subsequently found that, even when substantial color remained after adding water, a blunting of the curve near the maximum sometimes resulted. It is now believed that dilution with water should be avoided with the substituted benzaldehydes, although such procedure may result in the maxima occurring at 2  $m\mu$  or so longer wave length in some instances.

**Development of Color with "Native" and Other Soluble Lignins.**—The procedure used was similar to that with the cinnamaldehydes. Thus 0.0025 g. of spruce "native" lignin was moistened with a drop of water and 1.5 cc. of 0.001 molar ethanol solution of resorcinol added. After a few minutes agitation, 5 cc. of a 38% solution of hydrogen chloride in ethanol was introduced and the mixture allowed to stand 10 minutes at room temperature. It was then diluted to 15 cc. with 3.8 cc. of water and the balance ethanol, cooling under the tap.

With thioglycolic acid lignin no water was added, and sufficient dioxane was introduced to maintain a clear solution.

**Measurement of Color.**—The absorption spectrum was measured with a Beckman model DU spectrophotometer adjusted for near maximum resolution. Since at ordinary temperatures fading was often troublesome, the colored

(25) M. Scholtz and A. Wiedemann, *Ber.*, **36**, 853 (1903).

(26) H. Pauley and K. Feuerstein, *ibid.*, **62B**, 303 (1929).

(27) K. Feuerstein, *J. prakt. Chem.*, **143**, 174 (1935).

(28) H. Pauly and K. Wascher, *Ber.*, **56B**, 603 (1923).

(29) H. Pauly and L. Strassberger, *ibid.*, **62B**, 2277 (1929).

(30) K. W. Rosenmund, *ibid.*, **51**, 594 (1918).

(31) I. A. Pearl, *THIS JOURNAL*, **70**, 1746 (1948).

(32) L. Claisen and O. Eisleb, *Ann.*, **401**, 21 (1913).

(33) K. Freudenberg and H. Richtzenhain, *Ber.*, **76B**, 997 (1943).

TABLE III  
 NEW COMPOUNDS

Compounds	M. p., °C. <sup>a</sup>	Formula	Carbon, %		Hydrogen, %	
			Calcd.	Found	Calcd.	Found
Flavanones						
5,7-Dihydroxy-3',4'-dimethoxy	209-210	C <sub>17</sub> H <sub>16</sub> O <sub>6</sub>	64.53	64.75	5.10	5.21
7-Hydroxy-3'-methoxy-4'-ethoxy	195-196 (dec.)	C <sub>18</sub> H <sub>12</sub> O <sub>6</sub>	68.75	68.97	5.77	5.90
7-Hydroxy-3'-methoxy-4'-isopropoxy	129-131	C <sub>19</sub> H <sub>20</sub> O <sub>6</sub>	69.48	69.75	6.14	6.10
3',4',5',7-Tetrahydroxy	<sup>b</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	62.48	62.12	4.20	4.58
4',7-Dihydroxy-3',5'-dimethoxy	171-173 (dec.)	C <sub>17</sub> H <sub>16</sub> O <sub>6</sub>	64.53	64.22	5.10	5.18
4',7-Hydroxy-3'-methoxy-5'-propenyl	200-202 (dec.)	C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>	69.91	69.61	5.56	5.67
Chalcones						
2',4'-Dihydroxy-3-methoxy-4-ethoxy	198-200	C <sub>18</sub> H <sub>12</sub> O <sub>5</sub>	68.75	68.70	5.77	5.94
2',4'-Dihydroxy-3-methoxy-4-isopropoxy	176-177	C <sub>19</sub> H <sub>20</sub> O <sub>5</sub>	69.48	69.63	6.14	6.12
2',3,4,4',5-Pentahydroxy	<sup>c</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	62.48	62.44	4.20	4.38
2',4,4'-Trihydroxy-3,5-dimethoxy	234-236	C <sub>17</sub> H <sub>16</sub> O <sub>6</sub>	64.53	64.81	5.10	5.19
2',4,4'-Trihydroxy-3-methoxy-5-propenyl	203-205	C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>	69.91	70.18	5.56	5.56
Substituted benzaldehydes						
2-Methoxy-3-isopropoxy	<sup>d</sup>	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	68.00	68.21	7.27	7.44
4-Hydroxy-3-methoxy-5-propenyl	98-99	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>	68.71	68.79	6.30	6.31
Substituted cinnamaldehydes						
3-Methoxy-4-isopropoxy	92-93	C <sub>15</sub> H <sub>16</sub> O <sub>3</sub>	70.87	70.51	7.33	7.33
3-Methoxy-2-isopropoxy	39-40	C <sub>15</sub> H <sub>16</sub> O <sub>3</sub>	70.87	70.90	7.33	7.29

<sup>a</sup> All melting points corrected. <sup>b</sup> Becomes yellow at about 150°, then olive, and sinters to a black semi-liquid at 280°  
<sup>c</sup> Becomes brown at about 250° and sinters to a black semi-liquid at 290°. <sup>d</sup> Boiling point 155-158° at 18 mm.

solutions were cooled in ice-water immediately after the development of the color, and readings were made in a room maintained at 4° in which the instrument and accessories were kept. In the data recorded, the blank was a mixture of 5 cc. of concentrated hydrochloric acid and 10 cc. of 95% ethanol.

**Absorption Spectra of Wood Sections.**—Sections were cut from green sapwood at an angle of 45° with the fiber direction and so that they measured 12 mm. in the tangential direction and 25 mm. in the radial direction. The spruce sections were cut 100 microns thick and the aspen 120 microns. Sections were stained by immersing them in a 0.1 molar solution of resorcinol in 50% aqueous alcohol for 10 minutes and then in a 20% solution of hydrogen chloride in 95% ethanol for 10 minutes. The sections were blotted and mounted on a microscope slide with a cover glass, using  $\beta$ -phenylethyl alcohol as a mounting medium. A similar but unstained section was mounted on the same slide, and the slide was placed in a wooden holder designed for the purpose. When in the instrument the annual growth rings of the wood were at right angles to the slit direction. Readings were made as with solutions.

In the development of color with the aldehydes, soluble lignins and wood, a variety of conditions were investigated but, though the intensity of color varied widely no substantial displacement of the absorption maximum was observed.

**Acknowledgment.**—The author wishes to thank Dr. D. E. Bland, Australian Forest Products Laboratories, for preparing the insignis pine methanol lignin; Dr. F. E. Brauns, Institute of Paper Chemistry, for samples of spruce and aspen native lignin; Dr. K. Freudenberg, Heidelberg University, for the sample of enzymatically polymerized coniferyl alcohol; Dr. F. F. Nord, Fordham University for Scotch pine lignins; and Dr. C. R. Russell, Northern Regional Research Laboratory, U. S. Department of Agriculture, for the sample of 2-hydroxy-3-methoxy-5-formylacetophenone and polymer from vanillin acetate.

MADISON, WIS.

RECEIVED AUGUST 25, 1950